5   PARTICLE DELIVERY TECHNIQUES

Cross-Reference to Related Applications
            This application is a continuation-in-part of
International Patent Application Numbers PCT/GB97/01636,
10   filed June 17, 1997, and PCT/GB97/02478, filed September
11, 1997, both designating the United States, from which
applications priority is claimed pursuant to 35 U.S.C.
§365(c) and which applications are incorporated herein by
reference in their entireties.

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Technical Field
            The present invention relates generally to
particle delivery methods. More particularly, the
invention pertains to *in vivo* and *ex vivo* delivery of
20   powdered nucleic acid molecules into mammalian tissue
using needleless injection techniques. The invention
also relates to methods for forming dense, substantially
solid particles from non-dense particulate pharmaceutical
compositions such as those prepared using freeze-drying
25   or spray drying techniques. The densified compositions
obtained using the method are particularly suitable for
transdermal particle delivery from a needleless syringe
system.

30   Background of the Invention
            The ability to deliver agents into and through
skin surfaces (transdermal delivery) provides many
advantages over oral or other parenteral delivery
techniques. In particular, transdermal delivery provides
35   a safe, convenient and noninvasive alternative to

traditional drug administration systems, conveniently avoiding the major problems associated with oral delivery, e.g., variable rates of absorption and metabolism, gastrointestinal irritation and/or bitter or

5   unpleasant drug tastes. Transdermal delivery also avoids problems associated with traditional needle and syringe delivery, e.g., needle pain, the risk of introducing infection to treated individuals, the risk of contamination or infection of health care workers caused

10   by accidental needle-sticks and the disposal of used needles. In addition, such delivery affords a high degree of control over blood concentrations of administered drugs.

       However, despite its clear advantages,

15   transdermal drug delivery presents a number of its own inherent logistical problems. The passive delivery of drugs through intact skin necessarily entails the transport of molecules through a number of structurally different tissues, including the stratum corneum, the

20   viable epidermis, the papillary dermis, and the capillary walls in order for the drug to gain entry into the blood or lymph system. Transdermal delivery systems must therefore be able to overcome the various resistances presented by each type of tissue. In light of the above,

25   a number of alternatives to passive transdermal delivery have been developed. These alternatives include the use of skin penetration enhancing agents, or "permeation enhancers," to increase skin permeability, as well as non-chemical modes such as the use of iontophoresis,

30   electroporation or ultrasound. However, such techniques often give rise to unwanted side effects, such as skin irritation or sensitization. Thus, the number of drugs that can be safely and effectively administered using traditional transdermal delivery methods has remained

35   limited.

More recently, a novel transdermal delivery
system that entails the use of a needleless syringe to
fire solid drug-containing particles in controlled doses
into and through intact skin has been described.   In

5   particular, commonly owned U.S. Patent No. 5,630,796 to
Bellhouse et al. describes a needleless syringe that
delivers pharmaceutical particles entrained in a
supersonic gas flow.   The needleless syringe is used for
transdermal delivery of powdered drug compounds and

10   compositions, for delivery of genetic material into
living cells (e.g., gene therapy) or nucleic acid
immunization, and for the delivery of biopharmaceuticals
to skin, muscle, blood or lymph.   The needleless syringe
can also be used in conjunction with surgery to deliver

15   drugs and biologics to organ surfaces, solid tumors
and/or to surgical cavities (e.g., tumor beds or cavities
after tumor resection).   In theory, practically any
pharmaceutical agent that can be prepared in a
substantially solid, particulate form can be safely and

20   easily delivered using such devices.

One particular needleless syringe generally
comprises an elongate tubular nozzle having a rupturable
membrane initially closing the passage through the nozzle
and arranged substantially adjacent to the upstream end

25   of the nozzle.   Particles of a therapeutic agent to be
delivered are disposed adjacent to the rupturable
membrane and are delivered using an energizing means
which applies a gaseous pressure to the upstream side of
the membrane sufficient to burst the membrane and produce

30   a supersonic gas flow (entraining the pharmaceutical
particles) through the nozzle for delivery from the
downstream end thereof.   The particles can thus be
delivered from the needleless syringe at delivery
velocities as high as Mach 1 to Mach 8, which velocities

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are readily obtainable upon the bursting of the
rupturable membrane.

Another needleless syringe configuration
generally includes the same elements as described above,
except that instead of having the pharmaceutical
particles entrained within a gas flow, the downstream end
of the nozzle is provided with a diaphragm which is
moveable between a resting "inverted" position (in which
the diaphragm presents a concavity on the downstream face
to contain the pharmaceutical particles) and an "everted"
position (in which the diaphragm is outwardly convex on
the downstream face as a result of a supersonic shockwave
having been applied to the upstream face of the
diaphragm). In this manner, the pharmaceutical particles
contained within the concavity of the diaphragm are
expelled at a high initial velocity from the device for
transdermal delivery thereof to a targeted tissue
surface.

Transdermal delivery using the above-described
needleless syringe configurations is carried out with
particles having an approximate size that generally
ranges between 0.1 and 250 μm. For drug delivery, a
typical particle size is usually at least about 10 to 15
μm (the size of a typical cell). For gene delivery, a
typical particle size is generally substantially smaller
than 10 μm. Particles larger than about 250 μm can also
be delivered from the device, with the upper limitation
being the point at which the size of the particles would
cause untoward damage to the skin cells. The actual
distance which the delivered particles will penetrate
depends upon particle size (e.g., the nominal particle
diameter assuming a roughly spherical particle geometry),
particle density, the initial velocity at which the
particle impacts the skin surface, and the density and
kinematic viscosity of the skin. In this regard, optimal

particle densities for use in needleless injection
generally range between about 0.1 and 25 g/cm^3,
preferably between about 0.8 and 1.5 g/cm^3, and injection
velocities generally range between about 150 and 3,000

5   m/sec.

A particularly unique feature of the needleless
syringe is the ability to optimize the depth of
penetration of delivered particles, thereby allowing for
targeted administration of pharmaceuticals to various

10   sites. For example, particle characteristics and/or
device operating parameters can be selected to provide
for penetration depths for, *inter alia*, epidermal or
dermal delivery. One approach entails the selection of
particle size, particle density and initial velocity to

15   provide a momentum density (e.g., particle momentum
divided by particle frontal area) of between about 2 and
10 kg/sec/m, and more preferably between about 4 and 7
kg/sec/m. Such control over momentum density allows for
tissue-selective delivery of the pharmaceutical

20   particles.

Accordingly, there is a need to provide a
reliable method for preparing sufficiently dense
particles (having a density of about 0.8 to 1.5 g/cm^3)
which have an average size of about 0.1 to 150 μm from a

25   wide variety of pharmaceutical compositions. These
pharmaceutical particles can thus be transdermally
delivered to a subject using a needleless syringe system.

Needleless syringes, such as those described
above, also provide a unique means for gene therapy and

30   nucleic acid immunization. These techniques provide for
the transfer of a desired gene into a subject with the
subsequent *in vivo* expression thereof. Gene transfer can
be accomplished by transfecting the subject's cells or
tissues *ex vivo* and reintroducing the transformed

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material into the host. Alternatively, genes can be administered directly to the recipient.

A number of methods have been developed for gene delivery in these contexts. For example, viral-based systems using, e.g., retrovirus, adenovirus, and adeno-associated viral vectors, have been developed for gene delivery. However, these systems pose the risk of delivery of replication-competent viruses. Hence, nonviral methods for direct transfer of genes into recipient cells and tissues are desirable.

Nonviral methods of gene transfer often rely on mechanisms employed by mammalian cells for the uptake and intracellular transport of macromolecules. For example, receptor-mediated methods of gene transfer have been developed. The technique utilizes complexes between plasmid DNA and polypeptide ligands that can be recognized by cell surface receptors. However, data suggests that this method may permit only transient expression of genes and thus has only limited application.

Additionally, microinjection techniques have been developed for the direct injection of genetic material into cells. The technique, however, is laborious and requires single cell manipulations. Thus, the method is inappropriate for use on a large scale.

Direct injection of DNA-containing solutions into the interstitial space for subsequent uptake by cells has also been described. For example, International Publication No. WO 90/11092, published 4 October 1990, describes the delivery of isolated polynucleotides to the interior of cells wherein the isolated polynucleotides are delivered into the interstitial space of the tissue and then taken up by individual cells to provide a therapeutic effect. Such methods entail the injection of the DNA-containing

solutions into tissue using conventional needles or cannulas, and are therefore not well suited for long term therapies or for field or home applications.

Biolistic particle delivery systems (particle bombardment systems) have also been developed for gene delivery into plant cells. Such techniques use a "gene gun" to introduce DNA-coated microparticles, such as DNA-coated metals, into cells at high velocities. The coated metals (biolistic core carriers) are generally propelled into cells using an explosive burst of an inert gas such as helium. See, e.g., U.S. Patent No. 5,100,792 to Sanford et al. The technique allows for the direct, intracellular delivery of small amounts of DNA.

Biolistic core carriers upon which the DNA is coated, such as tungsten, gold, platinum, ferrite, polystyrene or latex, have to date been needed to achieve adequate gene transfer frequency by such direct injection techniques. See, e.g., International Publication No. WO 94/23738, published October 27, 1994. In particular, these materials have been selected based on their availability in defined particle sizes around 1 μm in diameter, as well as providing a sufficiently high density to achieve the momentum required for cell wall or cell membrane penetration. Additionally, common biolistic core carriers are chemically inert to reduce the likelihood of explosive oxidation of fine microprojectile powders, are non-reactive with DNA and other components of the precipitating mixes, and display low toxicity to target cells. See e.g., *Particle Bombardment Technology for Gene Transfer*, (1994) Yang, N. ed., Oxford University Press, New York, NY pages 10-11.

However, such biolistic techniques are not appropriate for use with large DNA molecules since precipitation of such molecules onto core carriers can

lead to unstable configurations which will not withstand
the shear forces of gene gun delivery.

   Accordingly, there remains a need to provide a
highly efficient method for introducing therapeutically
5   relevant DNA or other nucleic acid molecules into
mammalian tissue cells wherein the method avoids the
problems commonly encountered with prior gene delivery
techniques.

10   Disclosure of the Invention

   The present invention is based on the
surprising discovery that substantially solid particles
of nucleic acid molecules having a nominal average
diameter of at least about 0.1 μm, preferably at least
15   about 10 μm (which are therefore larger than the average
mammalian cell), can be delivered into cells of mammalian
tissue without the need for biolistic core carriers. The
result is unexpected because it was heretofore believed
that only small DNA-coated core carrier particles, having
20   an extremely high particle density and a much smaller
size than a typical mammalian cell, could adequately be
used as microprojectiles in biolistic gene delivery
techniques. See e.g., *Particle Bombardment Technology
for Gene Transfer*, (1994) Yang, N. ed., Oxford University
25   Press, New York, NY pages 10-11.

   In the practice of the invention, powdered
nucleic acid molecules are delivered using needleless
injection techniques. In particular, a novel delivery
system that uses a needleless syringe to fire solid
30   particles of therapeutic agents in controlled doses into
and through intact skin has recently been described in
commonly owned U.S. Patent No. 5,630,796. The patent
describes a needleless syringe that delivers
pharmaceutical particles entrained in a high velocity gas
35   flow. The needleless syringe can be used for transdermal

delivery of powdered drug compounds and compositions, for delivery of genetic material into living cells (e.g., gene therapy) and for the delivery of biopharmaceuticals to skin, muscle, blood or lymph. The needleless syringe

5   can also be used in conjunction with surgery to deliver drugs and biologics to organ surfaces, solid tumors and/or to surgical cavities (e.g., tumor beds or cavities after tumor resection).

Furthermore, the nucleic acids to be delivered

10 can be converted from non-dense pharmaceutical powders or particulate formulations (e.g., those having particle densities below that required for transdermal delivery from a needleless syringe) into densified (compacted) particles that are optimally suited for transdermal

15 delivery using a needleless syringe. The method is equally applicable to densification of pharmaceutical agents other than nucleic acids.

Accordingly, in one embodiment, the invention is directed to a method for delivering solid particles

20 comprised of nucleic acid molecules to mammalian tissue for the genetic transformation of cells in the tissue with the delivered nucleic acids. In a substantial departure from conventional particle bombardment techniques, the nucleic acid particles transferred using

25 the method of the present invention are not delivered using biolistic core carriers. Furthermore, the molecules can have a particle size that is equal to or larger than the average mammalian cell size.

More particularly, densified particles

30 comprised of selected nucleic acid molecules and, optionally, suitable vehicles or excipients, are prepared for delivery to mammalian tissue via a needleless syringe which is capable of expelling the particles at delivery velocities approaching Mach 1 to Mach 8 speeds. The

35 particles have an average size that is at least about 0.1

μm, wherein an optimal particle size is usually at least about 10 to 15 μm (equal to or larger than the size of a typical mammalian cell). However, nucleic acid particles having average particle sizes of 250 μm or greater can
5   also be delivered using the present method. The depth that the delivered particles will penetrate the targeted tissue depends upon particle size (e.g., the nominal particle diameter assuming a roughly spherical particle geometry), particle density, the initial velocity at
10 which the particle impacts the tissue surface, and the density and kinematic viscosity of the tissue. In this regard, optimal individual particle densities (e.g., in contrast to bulk powder density) for use in needleless injection generally range between about 0.1 and 25 g/cm^3,
15 and injection velocities generally range between about 150 and 3,000 m/sec.

In various aspects of the invention, the above method can be practiced *in vivo* to provide targeted delivery of the nucleic acid particles to a target
20 tissue, such as delivery to the epidermis (for gene therapy applications) or to the *stratum basal* layer of skin (for nucleic acid immunization applications). In these aspects of the invention, particle characteristics and/or device operating parameters are selected to
25 provide optimal tissue-specific delivery. One particular approach entails the selection of particle size, particle density and initial velocity to provide a momentum density (e.g., particle momentum divided by particle frontal area) of between about 2 and 10 kg/sec/m, and
30 more preferably between about 4 and 7 kg/sec/m. Such control over momentum density allows for precisely controlled, tissue-selective delivery of the nucleic acid particles.

In other aspects of the invention, the
35 needleless syringe is used to transfect cells or tissues

ex vivo with the particulate nucleic acid molecules, wherein the transformed cells are subsequently reintroduced into the host.

In another embodiment of the invention, a

5 method is provided for converting non-dense pharmaceutical powders or particulate formulations (e.g., having density characteristics below that required for transdermal delivery from a needleless syringe) into densified (compacted) particles that are optimally suited

10 for transdermal delivery using a needleless syringe. Such particles have an optimal particle density ranging from about 0.1 to about 25 g/cm^3, preferably ranging from about 0.5 to about 3.0 g/cm^3, and most preferably ranging from about 0.8 to about 1.5 g/cm^3. The densified

15 particles are processed to obtain optimal particle sizes ranging from about 0.1 to about 250 μm, preferably ranging from about 0.1 to about 150 μm, and most preferably ranging from about 20 to about 60 μm. The method entails the compaction of a pharmaceutical

20 composition using high pressure and, optionally vacuum, to densify the composition. The resulting compacted material is then size-reduced using conventional methods to provide densified particles of optimized size.

In a related embodiment of the invention, a

25 method is provided for optimizing the density and particle size of a particulate pharmaceutical composition that has particle size and density characteristics that fall within the above ranges. These particles are rendered more suitable for needleless syringe delivery

30 using the above-described compaction and size-reduction techniques. In this manner, the penetration depths that are obtained when the optimized particles are delivered using a needleless syringe can be adjusted to provide targeted dermal or intra-dermal delivery.

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        In a further related embodiment, the invention
pertains to a method for optimizing the particle size and
density of a lyophilized or spray-dried biopharmaceutical
composition. The method entails the compaction of a
5   lyophilized or spray-dried pharmaceutical powder to
obtain a densified material. The densified material can
then be reground to produce compositions in which the
individual particles approach the theoretical maximum
density and are thus optimal for delivery by impact with
10   and penetration into the target tissue at high velocities
when delivered from a needleless syringe. In a
particular embodiment, lyophilized recombinant human
growth hormone (rhGH) powder is densified to obtain
particles in the range of about 20 to 50 μm and having a
15   bulk density of about 0.8 to 1.5 g/cc^3. The densified
rhGH particles are ideally suited for delivery from a
needleless syringe device.

        In another embodiment, the invention is
directed to a compacted particulate pharmaceutical
20   composition formed from a porous pharmaceutical
preparation. The compacted composition has an average
particle size in the range of 0.1 to 250 μm mean
diameter, a particle density in the range of 0.1 to 25
g/cm^3, and a bulk density of at least about 0.5 g/cc^3.
25   Needleless syringes comprising the compacted particulate
pharmaceutical preparation, as well as single-dose
containers for a needleless syringe comprising the same,
are also provided.

        In another embodiment, the invention is
30   directed to a method of delivering a selected
pharmaceutical agent to a vertebrate subject. The method
comprises providing a densified (compacted) particulate
pharmaceutical preparation as described above and
delivering the preparation to a target tissue of the
35   vertebrate subject by needleless syringe.

In yet a further embodiment, the invention is directed to particles of a suitable size and density for transdermal delivery by needleless injection, consisting of a gene construct and an excipient selected from the

5   group consisting of pharmaceutical grades of dextrose, sucrose, lactose, trehalose, mannitol, sorbitol, inositol, erythritol, dextrans, cyclodextrans, starch, cellulose, sodium or calcium phosphates, calcium sulfates, citric acid, tartaric acid, glycine, albumin,

10   gelatin, polyacrylates, high molecular weight polyethylene glycols, and combinations thereof.

These and other embodiments of the subject invention will readily occur to those of skill in the art in light of the disclosure herein.

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Brief Description of the Figures

Figure 1 is a pictorial representation of an *ex vivo* delivery apparatus having a needleless syringe arranged over a tissue culture plate containing cells to

20   be transformed with the particulate nucleic acid preparations described herein.

Sub 1

Figure 2 is a histogram depicting transformation efficiencies obtained using the apparatus of Figure 1 to deliver DNA particles at 30 bar pressure

25   over a 60 mm target distance as described in the Example.

Sub 2

Figure 3 is a graph depicting the transformation efficiencies obtained using the apparatus of Figure 1 to deliver DNA particles at 30 bar pressure over a range of target distances, also as described in

30   the Example.

Figure 4 depicts a comparison of the mean *in vivo* serum levels of recombinant human growth hormone (rhGH) in animals that were administered lyophilized rhGH powder by needleless injection (▲), densified rhGH

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particles (prepared by the method of the invention) by
needleless injection (■), or lyophilized rhGH powder by
sub-cutaneous injection (•).

5   <u>Detailed Description of the Preferred Embodiments</u>

The practice of the present invention will
employ, unless otherwise indicated, conventional methods
of molecular biology and recombinant DNA techniques
within the skill of the art. Such techniques are
10   explained fully in the literature. See, e.g., Sambrook,
et al., *Molecular Cloning: A Laboratory Manual* (2nd
Edition, 1989); Maniatis et al., *Molecular Cloning: A
Laboratory Manual* (1982); DNA Cloning: A Practical
Approach, vol. I & II (D. Glover, ed.); Perbal, A
15   Practical Guide to Molecular Cloning.

Before describing the present invention in
detail, it is to be understood that this invention is not
limited to particular pharmaceutical formulations or
process parameters as such may, of course, vary. It is
20   also to be understood that the terminology used herein is
for the purpose of describing particular embodiments of
the invention only, and is not intended to be limiting.

It must be noted that, as used in this
specification and the appended claims, the singular forms
25   "a", "an" and "the" include plural referents unless the
content clearly dictates otherwise. Thus, for example,
reference to "a nucleic acid molecule" includes a mixture
of two or more nucleic acid molecules, reference to "an
excipient" includes mixtures of two or more excipients,
30   and the like.

All publications, patents and patent
applications cited herein, whether *supra* or *infra*, are
hereby incorporated by reference in their entirety.

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A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art

5   to which the invention pertains. The following terms are intended to be defined as indicated below.

The term "transdermal" delivery captures both transdermal (or "percutaneous") and transmucosal administration, i.e., delivery by passage of a drug or

10   pharmaceutical agent through the skin or mucosal tissue. See, e.g., *Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); *Controlled Drug Delivery: Fundamentals and Applications*, Robinson and Lee (eds.),

15   Marcel Dekker Inc., (1987); and *Transdermal Delivery of Drugs*, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Aspects of the invention which are described herein in the context of "transdermal" delivery, unless otherwise specified, are meant to apply to both

20   transdermal and transmucosal delivery. That is, the compositions, systems, and methods of the invention, unless explicitly stated otherwise, should be presumed to be equally applicable to transdermal and transmucosal modes of delivery.

25   By "needleless syringe" is meant an instrument which delivers a particulate composition transdermally, without a conventional needle that pierces the skin. Needleless syringes for use with the present invention are discussed throughout this document.

30   As used herein, the term "drug" or "pharmaceutical agent" intends any compound or composition of matter which, when administered to an organism (human or animal) induces a desired pharmacologic and/or physiologic effect by local and/or

35   systemic action. The term therefore encompasses those

compounds or chemicals traditionally regarded as drugs,
vaccines, as well as biopharmaceuticals including
molecules such as peptides, hormones, nucleic acids, gene
constructs and the like. More particularly, the term

5   "drug" or "pharmaceutical agent" includes compounds or
compositions for use in all of the major therapeutic
areas including, but not limited to, anti-infectives such
as antibiotics and antiviral agents; analgesics and
analgesic combinations; local and general anesthetics;

10   anorexics; antiarthritics; antiasthmatic agents;
anticonvulsants; antidepressants; antihistamines; anti-
inflammatory agents; antinauseants; antineoplastics;
antipruritics; antipsychotics; antipyretics;
antispasmodics; cardiovascular preparations (including

15   calcium channel blockers, beta-blockers, beta-agonists
and antiarrythmics); antihypertensives; diuretics;
vasodilators; central nervous system stimulants; cough
and cold preparations; decongestants; diagnostics;
hormones; bone growth stimulants and bone resorption

20   inhibitors; immunosuppressives; muscle relaxants;
psychostimulants; sedatives; tranquilizers; proteins
peptides and fragments thereof (whether naturally
occurring, chemically synthesized or recombinantly
produced); and nucleic acid molecules (polymeric forms of

25   two or more nucleotides, either ribonucleotides (RNA) or
deoxyribonucleotides (DNA) including both double- and
single-stranded molecules, gene constructs, expression
vectors, antisense molecules and the like).
        The above drugs or pharmaceutical agents, alone

30   or in combination with other drugs or agents, are
typically prepared as pharmaceutical compositions which
can contain one or more added materials such as carriers,
vehicles, and/or excipients. The terms "carriers,"
"vehicles" and "excipients" are used interchangeably

35   herein and generally refer to substantially inert

materials which are nontoxic and do not interact with other components of the composition in a deleterious manner. However, these terms do not encompass biolistic core carriers. The terms capture materials that can be

5 used to increase the amount of solids in particulate pharmaceutical compositions, such as those prepared using spray-drying or lyophilization techniques. Examples of suitable carriers include water, silicone, gelatin, waxes, and like materials. Examples of normally employed

10 "excipients," include pharmaceutical grades of dextrose, sucrose, lactose, trehalose, erythritol, dextrans, cyclodextrans mannitol, sorbitol, inositol, starch, cellulose, sodium or calcium phosphates, calcium sulfate, citric acid, tartaric acid, glycine, albumin, gelatin,

15 polyacrylates high molecular weight polyethylene glycols (PEG), and combinations thereof.

"Gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in expression of non-integrated

20 transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells.

The nucleotide sequences are generally present

25 in a suitable nucleic acid molecule and delivered in the form of vectors. By "vector" is meant any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control

30 elements and which can transfer gene sequences between cells.

A "nucleotide sequence" or a "nucleic acid molecule" refers to DNA and RNA sequences. The term captures molecules that include any of the known base

35 analogues of DNA and RNA such as, but not limited to

4-acetylcytosine, 8-hydroxy-N6-methyladenosine,
aziridinylcytosine, pseudoisocytosine,
5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil,
5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil,

5   5-carboxymethylaminomethyluracil, dihydrouracil, inosine,
N6-isopentenyladenine, 1-methyladenine, 1-methylpseudo-
uracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-
guanine, 2-methyladenine, 2-methylguanine, 3-methyl-
cytosine, 5-methylcytosine, N6-methyladenine,

10   7-methylguanine, 5-methylaminomethyluracil, 5-methoxy-
aminomethyl-2-thiouracil, beta-D-mannosylqueosine,
5'-methoxycarbonylmethyluracil, 5-methoxyuracil,
2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic
acid methylester, uracil-5-oxyacetic acid, oxybutoxosine,

15   pseudouracil, queosine, 2-thiocytosine, 5-methyl-
2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,
N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic
acid, pseudouracil, queosine, 2-thiocytosine, and
2,6-diaminopurine.

20   A "coding sequence" or a sequence which
"encodes" a particular polypeptide, is a nucleic acid
sequence which is transcribed (in the case of DNA) and
translated (in the case of mRNA) into a polypeptide *in
vitro* or *in vivo* when placed under the control of

25   appropriate regulatory sequences. The boundaries of the
coding sequence are conventionally determined by a start
codon at the 5' (amino) terminus and a translation stop
codon at the 3' (carboxy) terminus. A coding sequence
can include, but is not limited to, cDNA from procaryotic

30   or eukaryotic mRNA, genomic DNA sequences from
procaryotic or eukaryotic DNA, and even synthetic DNA
sequences. A transcription termination sequence will
usually be located 3' to the coding sequence.
            The term DNA "control sequences" refers

35   collectively to promoter sequences, polyadenylation

signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication,

5   transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected gene is capable of being replicated, transcribed and translated in an appropriate recipient cell.

10   "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding

15   sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding

20   sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

             By "isolated" when referring to a nucleotide sequence, or a nucleic acid molecule containing the nucleotide sequence, is meant that the indicated molecule

25   is present in the substantial absence of other biological macromolecules of the same type. Thus, an "isolated nucleic acid molecule which encodes a particular polypeptide" refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that

30   do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

             The term "transfection" is used to refer to the

35   uptake of foreign DNA by a host cell, and a host cell has

been "transformed" as a result of having been transfected. The foreign DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. By "host cell," or "host

5   mammalian cell" is meant a cell which has been transfected, or is capable of being transfected, by a nucleic acid molecule containing a nucleotide sequence of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in

10   morphology or in genetic make-up to the original parent, so long as the nucleotide sequence of interest is present within the cell.

By "biolistic core carrier" is meant a carrier on which a nucleic acid (e.g., DNA) is coated in order to

15   impart a defined particle size as well as a sufficiently high density to achieve the momentum required for cell wall penetration, such that the DNA can be delivered using biolistic techniques, such as by use of a gene gun (see, e.g., U.S. Patent No. 5,100,792). Biolistic core

20   carriers typically include dense solids such as tungsten, gold, platinum, ferrite, polystyrene and latex. See e.g., *Particle Bombardment Technology for Gene Transfer*, (1994) Yang, N. ed., Oxford University Press, New York, NY pages 10-11.

25   By "vertebrate subject" is meant any member of the subphylum cordata, particularly mammals, including, without limitation, humans and other primates. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered.

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B. Modes of Carrying Out the Invention

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters

35   as such may, of course, vary. It is also to be

understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

5   Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

As explained above, the present invention allows for the highly efficient delivery of solid

10   particles of nucleic acid molecules having a nominal average diameter of at least about 0.1 μm, preferably at least about 10 μm, to mammalian tissues. The method utilizes biolistic gene transfer techniques yet surprisingly allows for the delivery of nucleic acid

15   molecules without the need for biolistic core carriers.

A wide variety of nucleic acid molecules can be delivered using the methods of the invention. Generally, the molecules contain coding regions with suitable control sequences or other therapeutically relevant

20   nucleotide sequences. The nucleic acid molecules are prepared in the form of vectors which include the necessary elements to direct transcription and translation in a host cell. If expression is desired using the host's enzymes (such as by the use of

25   endogenous RNA polymerase), the gene or genes will be present in the vectors operatively linked to control sequences recognized by the particular host, or even particular cells within the host. Thus, eucaryotic and phage control elements will be present for expression in

30   mammalian hosts. Such sequences are known in the art and are discussed more fully below.

Suitable nucleotide sequences for use in the delivery methods of the present invention include any therapeutically relevant nucleotide sequence. Thus, the

35   present invention can be used to deliver one or more

genes encoding a protein defective or missing from a target cell genome or one or more genes that encode a non-native protein having a desired biological or therapeutic effect (e.g., an antiviral function). The invention can also be used to deliver a nucleotide sequence capable of providing immunity, for example an immunogenic sequence that serves to elicit a humoral and/or cellular response in a subject, or a sequence that corresponds to a molecule having an antisense or ribozyme function.

Suitable genes which can be delivered include those used for the treatment of inflammatory diseases, autoimmune, chronic and infectious diseases, including such disorders as AIDS, cancer, neurological diseases, cardiovascular disease, hypercholestemia; various blood disorders including various anemias, thalassemia and hemophilia; genetic defects such as cystic fibrosis, Gaucher's Disease, adenosine deaminase (ADA) deficiency, emphysema, etc. A number of antisense oligonucleotides (e.g., short oligonucleotides complementary to sequences around the translational initiation site (AUG codon) of an mRNA) that are useful in antisense therapy for cancer and for viral diseases have been described in the art. See, e.g., Han et al. (1991) *Proc. Natl. Acad. Sci. USA* <u>88</u>:4313; Uhlmann et al. (1990) *Chem. Rev.* <u>90</u>:543; Helene et al. (1990) *Biochim. Biophys. Acta.* <u>1049</u>:99; Agarwal et al. (1988) *Proc. Natl. Acad. Sci. USA* <u>85</u>:7079; and Heikkila et al. (1987) *Nature* <u>328</u>:445. A number of ribozymes suitable for use herein have also been described. See, e.g., Cech et al. (1992) *J. Biol. Chem.* <u>267</u>:17479 and U.S. Patent No. 5,225,347 to Goldberg et al.

For example, in methods for the treatment of solid tumors, genes encoding toxic peptides (e.g., chemotherapeutic agents such as ricin, diphtheria toxin

and cobra venom factor), tumor suppressor genes such as p53, genes coding for mRNA sequences which are antisense to transforming oncogenes, antineoplastic peptides such as tumor necrosis factor (TNF) and other cytokines, or transdominant negative mutants of transforming oncogenes, can be delivered for expression at or near the tumor site.

Similarly, genes coding for peptides known to display antiviral and/or antibacterial activity, or stimulate the host's immune system, can also be administered. Thus, genes encoding many of the various cytokines (or functional fragments thereof), such as the interleukins, interferons, and colony stimulating factors, will find use with the instant invention. The gene sequences for a number of these substances are known.

For the treatment of genetic disorders, functional genes corresponding to genes known to be deficient in the particular disorder can be administered to the subject. The instant methods will also find use in antisense therapy, e.g., for the delivery of oligonucleotides able to hybridize to specific complementary sequences thereby inhibiting the transcription and/or translation of these sequences. Thus, DNA or RNA coding for proteins necessary for the progress of a particular disease can be targeted, thereby disrupting the disease process. Antisense therapy, and numerous oligonucleotides which are capable of binding specifically and predictably to certain nucleic acid target sequences in order to inhibit or modulate the expression of disease-causing genes are known and readily available to the skilled practitioner. Uhlmann et al. (1990) *Chem. Rev.* 90:543, Neckers et al (1992) *Crit. Rev. Oncogenesis* 3:175; Simons et al. (1992) *Nature* 359:67; Bayever et al. (1992) *Antisense Res. Dev.* 2:109;

Whitesell et al. (1991) *Antisense Res. Dev.* 1:343; Cook
et al. (1991) *Anti-Cancer Drug Design* 6:585; Eguchi et
al. (1991) *Annu. Rev. Biochem.* 60:631. Accordingly,
antisense oligonucleotides capable of selectively binding
5   to target sequences in host cells are provided herein for
use in antisense therapeutics.

For nucleic acid immunizations, antigen-
encoding expression vectors can be delivered to a subject
for the purpose of eliciting humoral and/or cellular
10   immune responses to antigens encoded by the vector. In
particular, humoral, cytotoxic cellular and protective
immune responses elicited by direct intramuscular
injection of antigen-encoding DNAs have been described.
Tang et al. (1992) *Nature* 358:152; Davis et al. (1993)
15   Hum. Molec. Genet. 2:1847; Ulmer et al. (1993) *Science*
258:1745; Wang et al. (1993) *Proc. Natl. Acad. Sci. USA*
90:4156; Eisenbraun et al. (1993) *DNA Cell Biol.* 12:791;
Fynan et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:12476;
Fuller et al. (1994) *AIDS Res. Human Retrovir.* 10:1433;
20   and Raz et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:9519.
In addition, these immune responses have also been
elicited using biolistic techniques. See, e.g, EP
0500799.

25   Isolation of Genes and Construction of Vectors:
Nucleotide sequences selected for use in the
present invention can be derived from known sources, for
example, by isolating the same from cells containing a
desired gene or nucleotide sequence using standard
30   techniques. Similarly, the nucleotide sequences can be
generated synthetically using standard modes of
polynucleotide synthesis that are well known in the art.
See, e.g., Edge et al. (1981) *Nature* 292:756; Nambair et
al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol.*
35   *Chem.* 259:6311. Generally, synthetic oligonucleotides

can be prepared by either the phosphotriester method as
described by Edge et al. (*supra*) and Duckworth et al.
(1981) *Nucleic Acids Res.* 9:1691, or the phosphoramidite
method as described by Beaucage et al. (1981) *Tet. Letts.*
5   22:1859, and Matteucci et al. (1981) *J. Am. Chem. Soc.*
103:3185. Synthetic oligonucleotides can also be
prepared using commercially available automated
oligonucleotide synthesizers. The nucleotide sequences
can thus be designed with appropriate codons for a
10   particular amino acid sequence. In general, one will
select preferred codons for expression in the intended
host. The complete sequence is assembled from
overlapping oligonucleotides prepared by standard methods
and assembled into a complete coding sequence. *See*,
15   *e.g.*, Edge et al. (*supra*); Nambair et al. (*supra*) and Jay
et al. (*supra*).

        A particularly convenient method for obtaining
nucleic acid sequences for use herein is by recombinant
means. Thus, a desired nucleotide sequence can be
20   excised from a plasmid carrying the same using standard
restriction enzymes and procedures. Site specific DNA
cleavage is performed by treating with the suitable
restriction enzyme (or enzymes) under conditions which
are generally understood in the art, and the particulars
25   of which are specified by manufacturers of commercially
available restriction enzymes. If desired, size
separation of the cleaved fragments may be performed by
polyacrylamide gel or agarose gel electrophoresis using
standard techniques.

30   Restriction cleaved fragments may be blunt
ended by treating with the large fragment of *E. coli* DNA
polymerase I (Klenow) in the presence of the four
deoxynucleotide triphosphates (dNTPs) using standard
techniques. The Klenow fragment fills in at 5'
35   single-stranded overhangs but digests protruding 3'

single strands, even though the four dNTPs are present.
If desired, selective repair can be performed by
supplying only one, or several, selected dNTPs within the
limitations dictated by the nature of the overhang.

5   After Klenow treatment, the mixture can be extracted with
e.g. phenol/chloroform, and ethanol precipitated.
Treatment under appropriate conditions with S1 nuclease
or BAL-31 results in hydrolysis of any single-stranded
portion.

10   PCR techniques can also be used in order to
obtain a nucleic acid molecule of interest. Generally,
the technique involves amplification of sequences from a
human genomic or cDNA library. Degenerate or
nondegenerate oligonucleotide primers for PCR may be

15   prepared based on known amino acid sequences or on
sequences of homologous genes. The products of such PCR
reactions may be selected according to size by gel
electrophoresis. Such PCR methods are described in e.g.,
U.S. Patent Nos. 4,965,188; 4,800,159; 4,683,202;

20   4,683,195.
             Once coding sequences for desired peptides or
proteins have been prepared or isolated, such sequences
can be cloned into any suitable vector or replicon.
Numerous cloning vectors are known to those of skill in

25   the art, and the selection of an appropriate cloning
vector is a matter of choice. Ligations to other
sequences are performed using standard procedures, known
in the art.
             Selected nucleotide sequences can be placed

30   under the control of regulatory sequences such as a
promoter, ribosome binding site and, optionally, an
operator (collectively referred to herein as "control"
elements), so that the sequence encoding the desired
protein is transcribed into RNA in the host tissue

35   transformed by a vector containing this expression

construct.   The coding sequence may or may not contain a
signal peptide or leader sequence.

The choice of control elements will depend on
the host being transformed and the type of preparation

5   used.   Thus, if the host's endogenous transcription and
translation machinery will be used to express the
proteins, control elements compatible with the particular
host will be utilized.   In this regard, several promoters
for use in mammalian systems are known in the art and

10   include, but are not limited to, promoters derived from
SV40, CMV, HSV, RSV, MMTV, T7, T3, among others.
Similarly, promoters useful with procaryotic enzymes are
known and include the tac, spa, trp, trp-lac λ-p_L, T7,
phoA promoters, as well as others.

15   In addition to control sequences, it may be
desirable to add regulatory sequences which allow for
regulation of the expression of protein sequences encoded
by the delivered nucleotide sequences.   Regulatory
sequences are known to those of skill in the art, and

20   examples include those which cause the expression of a
coding sequence to be turned on or off in response to a
chemical or physical stimulus, including the presence of
a regulatory compound.   Other types of regulatory
elements may also be present in the vector, for example,

25   enhancer sequences.

An expression vector is constructed so that the
particular coding sequence is located in the vector with
the appropriate control and, optionally, regulatory
sequences such that the positioning and orientation of

30   the coding sequence with respect to the control sequences
allows the coding sequence to be transcribed under the
"control" of the control sequences (i.e., RNA polymerase
which binds to the DNA molecule at the control sequences
transcribes the coding sequence).   Modification of the

35   sequences encoding the particular protein of interest may

be desirable to achieve this end. For example, in some
cases it may be necessary to modify the sequence so that
it is attached to the control sequences with the ap-
propriate orientation; i.e., to maintain the reading

5   frame. The control sequences and other regulatory
sequences may be ligated to the coding sequence prior to
insertion into a vector. Alternatively, the coding
sequence can be cloned directly into an expression vector
which already contains the control sequences and an

10   appropriate restriction site.

Preparation of Particulate Molecules:
         Once obtained and/or constructed, the nucleic
acid molecules are prepared for delivery in particulate

15   form. For example, particulate molecules can be produced
using particle formation techniques well known in the
art, such as but not limited to spray-drying, spray-
coating, freeze-drying (lyophilization) and super
critical fluid precipitation.

20   In one embodiment, the invention entails a
procedure for forming dense particles from low density
particulate pharmaceutical preparations. In particular,
manufacturing processes for preparing pharmaceutical
particles from delicate molecules such as DNA, proteins

25   or peptides generally result in low density particles
having either a hollow spherical or open lattice
monolithic structure. Such particles are poorly suited
for use in needleless syringe delivery systems, wherein
the particles must have sufficient physical strength to

30   withstand sudden acceleration to velocities approaching
the speed of sound and the impact with, and passage
through, the skin and tissue.
         One common method of preparing particulate
biopharmaceuticals, such as nucleic acids, is

35   lyophilization (freeze-drying). Lyophilization relates

to a technique for removing moisture from a material and
involves rapid freezing at a very low temperature,
followed by rapid dehydration by sublimation in a high
vacuum. This technique typically yields low-density

5   porous particles having an open matrix structure. Such
particles are chemically stable, but are rapidly
reconstituted (disintegrated and/or brought into
solution) when introduced into an aqueous environment.
Another method of providing particulate

10   preparations that can be used with these and other
delicate or heat-sensitive biomolecules is spray-drying.
Spray-drying relates to the atomization of a solution of
one or more solids using a nozzle, spinning disk or other
device, followed by evaporation of the solvent from the

15   droplets. More particularly, spray-drying involves
combining a highly dispersed liquid preparation (e.g., a
solution, slurry, emulsion or the like) with a suitable
volume of hot air to produce evaporation and drying of
the liquid droplets. Spray-dried pharmaceuticals are

20   generally characterized as homogenous spherical particles
that are frequently hollow. Such particles have low
density and exhibit a rapid rate of solution.
The low-density particulate solids produced by
lyophilization and spray-drying techniques are ideal for

25   redissolution for parenteral administration in solution
via syringe or catheters. However, such particles are
not useful for delivery from a needleless syringe in a
solid form. Accordingly, for purposes of the present
method, the preparations are densified to provide

30   particles including nucleic acid molecules that are much
better suited for delivery using a needleless syringe
(e.g., substantially solid particles having a size of
about 50 μm and a density of at least about 0.9 to 1.5
g/cc^3). In particular, the open lattice or hollow shell

35   particles provided by spray-drying or lyophilization can

be condensed without heating or shear to provide dense materials that can be milled or otherwise size-reduced to yield pharmaceutical particles having both size and density characteristics suitable for delivery by

5   needleless injection.

The nucleic acids for delivery by the method of the invention, may be initially prepared in a formulation suitable for spray-drying or lyophilization. Such formulations generally require only a solution in which

10   the nucleic acids will be stable for freezing and lyophilization and, optionally, an excipient for the drying procedure which is acceptable for parenteral delivery. In this regard, suitable excipients may be added to the formulations to provide sufficient mass for

15   an individual dose, enabling measurement of doses by practical processes, e.g., by weight or volume. Typical dosages can be about 0.5 to about 5 mg, preferably about 1 to about 2 mg. Suitable excipients include, but are not limited to, carbohydrates (such as trehalose,

20   glucose, dextrose and sucrose) or polyols (such as mannitol). Amino acids such as glycine and its hydrochloride salt can be used as buffers as well as phosphate, lactate or citrate buffers, among others. Additionally, any known composition for DNA stabilization

25   will find use in the present formulations. The compositions may optionally include additive agents such as cryoprotectants, antioxidants, or the like. Adjusting compositions to enhance physical and chemical stability of the various particulate nucleic acid

30   formulations provided herein is within the ordinary skill in the art.

One particular approach to stabilization during reprocessing of the nucleic acid formulations entails the use of additives which are combined with the solution

35   prior to freezing for lyophilization to cause the nucleic

acids to coil or ball and thus provide the genetic
material as a discontinuous phase in the otherwise
microscopically homogeneous particles. In such
formulations, the bulking agent would be the continuous
5   phase in the dried solid so that any grinding prior to
compression, compression densification and regrinding (as
described in detail below), and any particle attrition
during sizing via sieve or air classification,
acceleration and injection, would be less likely to
10   disrupt the long chain nucleic acids. Homogeneity of the
particles with respect to nucleic acid content is
critical because of the potential for segregation by size
during storage or injection.

          Condensing the nucleic acid powders can be
15   conducted by compaction in a suitable press (e.g., a
hydraulic press, tableting press or rotary press),
wherein the powders are compressed at about 1,000 to
24,000 pounds/square inch (e.g., 0.5 to 12 tons/square
inch or 7 to 170 MPa) for a suitable time. Compaction
20   can be carried out under vacuum if desired. The
resulting compacted material is then coarsely reground
until visually broken up. The particle size is then
reduced to about a 20 to 50 μm average size with an
optimal bulk particle density of around 0.9 to 1.5 g/cm^3,
25   or as close to absolute or theoretical density as
possible. Particle size reduction can be conducted using
methods well known in the art including, but not limited
to, roller milling, ball milling, hammer, air or impact
milling, attrition milling, sieving, sonicating, or
30   combinations thereof. The compression parameters and
particle sizing will, of course, vary depending upon the
starting material used, the desired target particle size
and density, and like considerations.

          Particle density can be ascertained using
35   helium pycnometry to measure absolute density and various

techniques to establish porosity such as mercury intrusion BET, flotation in a density gradient, and the like.   These techniques are all well known in the art.

Thus, the method can be used to obtain nucleic acid particles having a size ranging from about 10 to about 250 μm, preferably about 10 to about 150 μm, and most preferably about 20 to about 60 μm; and a particle density ranging from about 0.1 to about 25 g/cm^3, and a bulk density of about 0.5 to about 3.0 g/cm^3, or greater.

A particularly preferred method for providing nucleic acid molecules suitable for biolistic delivery is the novel densification/compaction technique described herein.   The technique is useful not only for preparing nucleic acid biopharmaceuticals, but can be used to prepare almost any desired physiologically active composition for needleless delivery.

Accordingly, in another embodiment of the invention, a method is provided for densifying (compacting) a non-nucleic acid pharmaceutical preparation.

As explained above, current manufacturing processes for preparing pharmaceutical particles from delicate molecules such as proteins or peptides are poorly suited for use in needleless syringe delivery systems.   For example, as discussed above, lyophilization typically yields low-density porous particles having an open matrix structure.   Exemplary biopharmaceuticals available as lyophilized particles include recombinant human growth factor (e.g., Genotropin®, Pharmacia, Piscataway, NJ); somatrem (e.g., Protropin®, Genentech, S. San Francisco, CA); somatropin (e.g., Humatrope®, Eli Lilly, Indianapolis, IN); recombinant interferon α-2a (e.g., Roferon®-A, Hoffman-La Roche, Nutley, NJ); recombinant interferon α-2b (e.g., Intron A®, Schering-

Plough, Madison, NJ); and recombinant alteplase (e.g., Activase®, Genentech, S. San Francisco, CA).

In addition, spray-dried pharmaceuticals are generally characterized as homogenous spherical particles that are frequently hollow. Such particles have low density and exhibit a rapid rate of solution. Exemplary heat-sensitive pharmaceuticals that are prepared using spray-drying techniques include the amino acids; antibiotics such as aureomycin, bacitracin, penicillin and streptomycin; ascorbic acid; cascara extracts; pepsin and similar enzymes; protein hydrolysates; and thiamine.

When spray-dried and lyophilized pharmaceutical particles are ground or milled, they yield very small, light and non-dense particles that are poorly suited for delivery through skin or mucosal tissues. In particular, such particles, when delivered from a needleless syringe, are often too light to have the momentum necessary to penetrate intact skin (e.g., pass through the stratum corneum) and would thus fail to enter the systemic circulation. In this regard, the stratum corneum is a thin layer of dense packed, highly keratinized cells, generally about 10-15 μm thick and which covers most of the human body. The stratum corneum thus provides the primary skin barrier which a transdermally-delivered particle must cross.

Accordingly, the present method entails densifying (compacting) such preparations to provide particles that are much better suited for delivery from a needleless syringe (e.g., substantially solid particles having a size of about 50 μm and a bulk density of at least about 0.5 to 1.5 g/cc^3). In particular, open lattice or hollow shell particles provided by spray-drying or lyophilization can be condensed without heating or shear to provide dense, compacted materials that can be milled or otherwise size-reduced to yield

pharmaceutical particles having both size and density
characteristics suitable for delivery by needleless
injection.

Condensing of the spray-dried or lyophilized
powders is typically conducted by compaction in a
suitable press (e.g., a hydraulic press, tableting press
or rotary press), wherein the powders are compressed at
about 1,000 to 24,000 pounds/square inch (0.5 to 12
tons/square inch) for a suitable time. This compaction
can be carried out under vacuum if desired. The
resulting compacted material is then coarsely reground
until visually broken up. The particle size is then
reduced to about a 20 to 50 μm average size to yield a
bulk density of around 0.5 to 1.5 g/cc^3 (with a particle
density of about 0.1 to 25 g/cm^3). Particle size
reduction can be conducted using methods well known in
the art including, but not limited to, roller milling,
ball milling, hammer or impact milling, attrition
milling, sieving, sonicating, or combinations thereof.
The compression parameters and particle sizing will, of
course, vary depending upon the starting material used,
the desired target particle size and density, and like
considerations. The starting material can be any
pharmaceutical preparation having a particle size and
density which one is desirous of changing to obtain more
optimal size and density characteristics for use in
needleless syringes.

Following densification, particles of suitable
size can be selected and classified using standard
techniques, known in the art, such as by vibratory, sonic
or jet sieving, cyclone separation, or like techniques,
well known in the art.

Actual particle density, or "absolute density,"
can be readily ascertained using known quantification
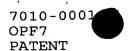techniques such as helium pycnometry and the like.

Alternatively, envelope density measurements can be used to assess suitable densification of the particulate pharmaceutical compositions. Envelope density information is useful in characterizing the density of porous objects of irregular size and shape. Envelope density, or "bulk density," is the mass of an object divided by its volume, where the volume includes that of its pores and small cavities. A number of methods of determining envelope density are known in the art, including wax immersion, mercury displacement, water absorption and apparent specific gravity techniques. A number of suitable devices are also available for determining envelope density, for example, the GeoPyc™ Model 1360, available from the Micromeritics Instrument Corp. The difference between the absolute density and envelope density of a sample pharmaceutical composition provides information about the sample's percentage total porosity and specific pore volume. In the practice of the invention, compaction of porous particulate pharmaceutical compositions will generally result in a reduction of porosity, and a concomitant increase in bulk (envelope) density.

Thus, the method can be used to obtain particles having a size ranging from about 0.1 to about 250 μm, preferably about 0.1 to about 150 μm, and most preferably about 20 to about 60 μm; a particle density ranging from about 0.1 to about 25 g/cm^3, and a bulk density of preferably about 0.5 to about 3.0 g/cm^3, and most preferably about 0.8 to about 1.5 g/cm^3.

The above-described method can also be used to optimize the density and particle size of a particulate pharmaceutical composition that has particle size and density characteristics that fall within the above ranges. In this manner, the penetration depths that are obtained when the optimized particles are delivered at

high velocities using a needleless syringe can be
adjusted to optimize targeted dermal or intra-dermal
delivery.

5   However, as noted hereinabove, the invention is
particularly suited for preparing densified particles
having optimized density from heat-sensitive
biopharmaceutical preparations of peptides, polypeptides,
proteins, nucleic acids and other such biological
molecules. Exemplary peptide and protein formulations
10   which can be densified using the instant method include,
without limitation, insulin; calcitonin; octreotide;
endorphin; liprecin; pituitary hormones (e.g., human
growth hormone and recombinant human growth hormone (hGH
and rhGH), HMG, desmopressin acetate, etc); follicle
15   luteoids; growth factors (such as growth factor releasing
factor (GFRF), somatostatin, somatotropin and platelet-
derived growth factor); asparaginase; chorionic
gonadotropin; corticotropin (ACTH); erythropoietin (EPO);
epoprostenol (platelet aggregation inhibitor); glucagon;
20   interferons; interleukins; menotropins (urofollitropin
follicle-stimulating hormone (FSH) and luteinizing
hormone (LH)); oxytocin; streptokinase; tissue
plasminogen activator (TPA); urokinase; vasopressin;
desmopressin; ACTH analogues; angiotensin II antagonists;
25   antidiuretic hormone agonists; bradykinin antagonists;
CD4 molecules; antibody molecules and antibody fragments
(e.g., Fab, Fab_2, Fv and sFv molecules); IGF-1;
neurotrophic factors; colony stimulating factors;
parathyroid hormone and agonists; parathyroid hormone
30   antagonists; prostaglandin antagonists; protein C;
protein S; renin inhibitors; thrombolytics; tumor
necrosis factor (TNF); vaccines (particularly peptide
vaccines including subunit and synthetic peptide
preparations); vasopressin antagonists analogues; and α-1

35

antitrypsin. Exemplary nucleic acid molecules are
detailed above.

Administration of the Particles:

5   Following formation, the particulate
preparations are delivered to mammalian tissue using a
needleless syringe. The compacted particles of the
present invention can be packaged in individual unit
dosages. As used herein, a "unit dosage" intends a
10   dosage receptacle containing a therapeutically effective
amount of a pharmaceutical powder produced according to
the methods of the present invention. The dosage
receptacle is generally one which fits within a
needleless syringe device to allow for transdermal
15   delivery from the device. Such receptacles can take the
form of capsules, foil pouches, sachets, cassettes, and
the like. Appropriate needleless syringes are described
herein above.

        Transdermal delivery from these various
20   needleless syringe configurations is carried out with
particles having an approximate size that generally
ranges between 0.1 and 250 μm. However, the optimal
particle size is usually at least about 10 to 15 μm (the
size of a typical cell). Particles larger than about 250
25   μm can also be delivered from the device, with the upper
limitation being the point at which the size of the
particles would cause untoward damage to the skin. Other
particulate biopharmaceuticals, such as peptide and
protein preparations, will generally have an approximate
30   size of 0.1 to about 250 μm, preferably about 0.1 to
about 150 μm, and most preferably about 20 to about 60
μm.

        The actual distance which the delivered
particles will penetrate depends upon particle size
35   (e.g., the nominal particle diameter assuming a roughly

spherical particle geometry), particle density, the
initial velocity at which the particle impacts the skin
surface, and the density and kinematic viscosity of the
skin. In this regard, optimal particle densities for use
5   in needleless injection generally range between about 0.1
and 25 g/cm^3, and optimal bulk densities range from about
0.5 and 3.0 g/cm^3. Injection velocities generally range
between about 100 and 3,000 m/sec.

Compositions containing a therapeutically
10   effective amount of the powdered molecules described
herein can be delivered to any suitable target tissue via
the above-described needleless syringes. For example,
the compositions can be delivered to muscle, skin, brain,
lung, liver, spleen, bone marrow, thymus, heart, lymph,
15   blood, bone cartilage, pancreas, kidney, gall bladder,
stomach, intestine, testis, ovary, uterus, rectum,
nervous system, eye, gland and connective tissues. For
nucleic acid molecules, delivery is preferably to, and
the molecules expressed in, terminally differentiated
20   cells; however, the molecules can also be delivered to
non-differentiated, or partially differentiated cells
such as stem cells of blood and skin fibroblasts.

The powdered compositions are administered to
the subject to be treated in a manner compatible with the
25   dosage formulation, and in an amount that will be
prophylactically and/or therapeutically effective. The
amount of the composition to be delivered, generally in
the range of from 0.5 μg/kg to 100 μg/kg of nucleic acid
molecule per dose, depends on the subject to be treated.
30   Doses for other pharmaceuticals, such as physiological
active peptides and proteins, generally range from about
0.1 μg to about 20 mg, preferably 10 μg to about 3 mg.
The exact amount necessary will vary depending on the age
and general condition of the individual to be treated,
35   the severity of the condition being treated, the

particular preparation delivered, the site of administration, as well as other factors. An appropriate effective amount can be readily determined by one of skill in the art.

5   Thus, a "therapeutically effective amount" of the present particulate compositions will be sufficient to bring about treatment or prevention of disease or condition symptoms, and will fall in a relatively broad range that can be determined through routine trials.

10

C. Experimental

        Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not
15   intended to limit the scope of the present invention in any way.

        Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should,
20   of course, be allowed for.

Example 1

        The following experiment was conducted to investigate the possibility of using freeze-dried DNA as
25   an alternative to DNA-coated metal particles in the biolistic transfer of genetic material. In particular, powdered DNA plasmids as well as DNA-coated tungsten particles as controls were delivered ex vivo to male human fibroblast HT1080 cells using a needleless syringe
30   apparatus as follows.

        Clone 123 is a small plasmid of ~11 kb which contains the β-galactosidase marker gene so that transient transformation can be measured with the chromogenic indicator X-Gal. Plasmids were bulked with a
35   carbohydrate excipient, trehalose. Trehalose was

selected as the excipient because of its stabilizing
properties (Colaco et al. (1992) *Bio/Technology* <u>10</u>:1009).
The trehalose was dissolved in distilled water and
filter-sterilized prior to adding the DNA to the

5   solution.   Three different solutions of DNA sugar were
made up with the proportions shown below in Table 1.

Table 1

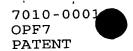| Preparation	1	2	3
Clone 123 (2.7μg/μL)	800 μg (296.3 μL)	160 μg (59.3 μL)	80 μg (29.6 μL)
Trehalose (100 mg/15 ml H_2O)	10 mg (1.5 ml)	10 mg (1.5 ml)	10 mg (1.5 ml)
Payload (for 8 μg DNA)	0.1 mg	0.5 mg	1.0 mg

The plasmid/sugar solutions were then freeze-dried (using
solid CO_2 and isopropanol to freeze the solution prior to
vacuum drying), and the freeze-dried DNA-trehalose solid
milled to form microparticles using an agate mortar and

20   pestle.

As a control, DNA-coated tungsten particles
were made by coating tungsten microprojectiles (19.35 x
10^3 kb.m^{-3}) of 1.014 μm median diameter (M-17,
GTE/Sylvania, Towanda, PA, USA) with forty micrograms of

25   Clone 123 plasmid DNA, giving five payloads of 8 μg DNA,
using a derivative of known methods for coating
microparticles. Potter et al. (1984) *Proc. Natl. Acad.
Sci. USA* <u>81</u>:7161, Klein et al. (1987) *Nature* <u>327</u>:70, and
Williams et al. (1991) *Proc. Natl. Acad. Sci. USA*

30   <u>88</u>:2726.

More particularly, prior to coating, the
tungsten particles were sterilized and brought into
suspension. A 50 mg sample of 1.048 μm (median diameter)
tungsten microprojectiles (M-17, GTE/Sylvania, Towanda,

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PA, USA) was weighed into a 1.5 cc Eppendorf tube and
then sterilized in 100% ethanol (EtOH). In order to
disperse the microprojectiles (disrupt particle
aggregates), the sterilized solution was sonicated

5   thoroughly by contacting the outside of the Eppendorf
tube with the probe of a sonicator. The dispersed
tungsten particles were centrifuged and the supernatant
removed. The tungsten particles were resuspended in 1 cc
sterile distilled water and centrifuged for two cycles,

10   and then stored in 1 cc sterile distilled water until
coating.

The plasmid DNA was absorbed to the tungsten
microprojectiles by adding 20 μL of the DNA (1 mg/mL) to
40 μL of the suspension of tungsten particles prepared

15   above. The suspension was vortexed to ensure adequate
mixing of the reagents. The following reagents were then
added, in the order given, with vortexing after each
addition: 253 μL CaCl$_2$ (2.5 M); 50 μL spermidine (0.10
M, stored frozen); and 207 μL sterile distilled H$_2$O. The

20   final mixture was vortexed for 10 minutes at 4 °C. The
DNA-coated tungsten microprojectiles were then
centrifuged at 500 G for 5 minutes. After
centrifugation, all supernatant was carefully removed and
100 μL of 70% EtOH added. The coated particles were

25   again centrifuged, all supernatant removed, and the final
preparation resuspended in 30 μL of 100% EtOH.

The above method resulted in suitable
quantities of DNA-coated tungsten particles to allow for
4 to 5 deliveries by the needleless syringe. The above-

30   noted reagent quantities can, of course, be varied to
provide different loadings of DNA in accordance with
known methods. The volume and molar concentration (M) of
the stock solutions used to coat tungsten with DNA are
given below in Table 2.

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Table 2

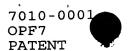| Component	Quantity (μL)
Tungsten particles (50 mg/mL)	40
Clone 123 (2.7 μg/μL)	14.8
Ca Cl$_2$ (2.5 M)	253
Spermidine (0.1 M)	50
distilled H$_2$O	212.2

For transformation, 6 cm diameter culture dishes were seeded with 5×10^5 male human fibroblast HT1080 cells 24 hours before transfection. Two replicate dishes were prepared for each of the treatments, and two negative control plates were also prepared.

The microparticles and the tungsten-coated particles were then delivered to cells using a needleless syringe as described above. The syringe included a 4.5 mL reservoir chamber with plunger type valve, a helium gas reservoir, a Mach 2 nozzle and 12 μm Mylar sheet hand-punched into 6 mm diameter diaphragms.

In particular, mylar diaphragms were first sterilized by singly layering between pieces of filter paper, stacked one atop the other, wrapped in aluminum foil and sealed completely with autoclave tape to ensure that no water entered the filter paper/diaphragm stack during the autoclave process. This was placed inside a beaker covered with aluminum foil and placed in an autoclave chamber.

Two 12 μm Mylar diaphragms of 6 mm diameter were used in the membrane cassette. One milligram and one-half milligram payloads of the freeze-dried DNA powder were loaded onto the lower membrane in the cassette. This payload was then covered with the other pieces of the cassette and the remaining diaphragm. Only preparations 2 and 3 were used in the experiment because

of the difficulty in weighing out small masses
accurately. Another five of the cassettes were each
loaded with 5 μL of the DNA/tungsten particle suspension.
All the above quantities gave a mass of about 8 μg of DNA
5   being delivered in each shot regardless of particle
formulation used.

Referring now to Figure 1, a delivery apparatus
2 was assembled which contained a needleless syringe 4
loaded with a cassette as described above. The
10   needleless syringe 4 was arranged on a ring stand 6 using
a standard tube clamp 8 to hold the syringe in position
relative to a culture dish 10 seeded with the HT1080
cells 12. The distance between the downstream terminus
14 of the needleless syringe 4 and the cells 14 in the
15   culture dish 10 was measured to affix a target distance,
generally indicated at d. In order to optimize the
parameters for delivery of the freeze-dried DNA, either
the target distance d was varied over a constant delivery
pressure, or the delivery pressure was varied over a
20   constant target distance. In particular, the DNA
preparations were fired from a target distance ranging
from 20 to 60 mm using helium driver gas pressures
ranging from 30 to 50 bar.

After transformation, cells were incubated for
25   2 days, stained, and then transient assays with X-gal
were performed to determine transformation efficiencies
using previously described methods. Murray, E.J. (ed)
(1991) *Methods in Molecular Biology: Gene Transfer and
Expression Protocols*, Vol. 7, Humana Press, Clifton, New
30   Jersey. Specifically, transformation efficiency was
assessed by counting the number of blue-stained cells.
The delivery parameters and transformation results are
depicted below in Table 3. Blast effect was rated from 1
point for quite small (diameter of dead cell zone being
35   approximately 5-8 mm) to five points for very large

(diameter of cell zone being greater than 30 mm). As can
be seen, transformation by the plasmid/trehalose powder
preparation of the present invention was on the same
order as that observed for the metallic particles.

5   As shown in Figure 2, optimal transformation
results were seen with the particulate plasmid/trehalose
preparation when delivered using 30 bar pressure at a
target distance of 60 mm. More particularly, Figure 2
provides a direct comparison of the transformation

10   efficiency obtained by delivery of the particulate
nucleic acid preparation (both preparations 2 and 3) with
historical and contemporary deliveries of DNA-coated
tungsten particles. Referring now to Figure 3, data
obtained for deliveries at 30 bar are depicted in a graph

15   which presents transformation efficiency as a function of
target distance. As can be seen, the optimal target
distance for the number 2 and 3 preparations (referred to
as OBS Formulation #2 and OBS Formulation #3,
respectively) was not reached; however, transformation

20   efficiency did substantially increase with increased
target distances. Further, when deliveries were carried
out at the maximum distance tested (60 mm),
transformation efficiencies obtained with the particulate
DNA formulations (#2 and #3) were appreciably better than

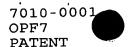25   those observed with the DNA-coated tungsten controls.

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Table 3

Shot	Formulation	Target Distance	Pressure	Blue Cell Count	Blast effect
A 1	Tungsten	60	30	224	2
A 2	Tungsten	60	30	596	2
A 3	Tungsten	60	30	575	2
A 4	Tungsten	60	30	581	2
A 5	Tungsten	60	30	-	-
B 1	#3 trehalose	20	30	155	3
B 2	#3 trehalose	20	30	227	3
C 1	#3 trehalose	40	30	654	2
C 2	#3 trehalose	40	30	643	2
D 1	#3 trehalose	40	50	394	4
D 2	#3 trehalose	40	50	175	5
E 1	#3 trehalose	60	30	1416	1
E 2	#3 trehalose	60	30	1654	1
F 1	#3 trehalose	60	50	408	3
F 2	#3 trehalose	60	50	486	3
G 1	#2 trehalose	20	30	166	3
G 2	#2 trehalose	20	30	180	3
H 1	#2 trehalose	20	50	129	4
H 2	#2 trehalose	20	50	53	4
J 1	#2 trehalose	40	30	347	2
J 2	#2 trehalose	40	30	546	2
K 1	#2 trehalose	40	50	377	4
K 2	#2 trehalose	40	50	198	4
L 1	#2 trehalose	60	30	1451	1
L 2	#2 trehalose	60	30	1164	1
M 1	#2 trehalose	60	50	409	3
M 2	#2 trehalose	60	50	336	3

Example 2

The following studies were carried out to assess the ability to deliver a powdered nucleic acid composition to test subjects *in vivo* using the methods of the invention.

Plasmid Vector Construct: The pGREEN-1 vector construct, which contains the Green Fluorescent Protein (GFP) gene under the control of a CMV promoter, was used so that gene expression could be assessed directly by UV microscopy of histological sections from treated tissue samples.

Powdered Nucleic Acid Compositions: A powdered nucleic acid composition was prepared as follows. A

mixture was formed by combining pGREEN-1 vector plasmid with trehalose sugar to obtain a 1μg:1mg (w/w) DNA-sugar composition. This composition was lyophilized, compressed, ground, and then sieved, using the techniques described hereinabove. The resulting condensed nucleic acid composition had an average particle size ranging from about 38-75 μm.

Administrations: C57BL/10 mice were treated with 1 mg of the particulate composition via needleless injection. The composition was delivered to a suitably prepared target skin surface, and histological sections were taken from the target site 24 hours after administration. GFP expression was determined directly using UV microscopy. As a result of the administrations, GFP expression was seen in the treated skin tissue, confirming successful *in vivo* delivery of the powdered nucleic acid composition to the target skin, and the subsequent transfection of host cells and expression of the GFP gene therefrom.

In another study, plasmids containing either a human Growth Hormone (hGH) or β-galactosidase (β-Gal) expression cassette were lyophilized with trehalose excipient to form nucleic acid formulations, which were compressed, ground, and then sieved, using the above-described techniques. The resulting condensed nucleic acid compositions had an average particle size ranging from about 38-75 μm.

Female pigs (weighing 20-25 kg) were anesthestised with halothane, and the belly skin was clipped to reveal a suitable target site. The above powdered nucleic acid compositions were individually administered to the prepared target site in 0.1 μg (hGH) or 1 μg (β-Gal) doses via a needleless injection device (delivery pressure of 60 bar). The target sites were biopsied 24 hours after treatment, and histological sections were

analyzed for human growth hormone or β-Gal expression. Although no hGH expression was seen within the detection limits of the assay, a moderate degree of β-Gal expression was seen in the treated sites. The lack of detectable hGH expression in this study is due, presumably, to the low loading density of the nucleic acid (0.1 μg) in the composition.

Example 3
Densification of Recombinant
Human Growth Hormone (rhGH)

Lyophilized recombinant human growth hormone powder (Genotropin®, available from Pharmacia, Piscataway, NJ) was obtained and reprocessed using the method of the invention. Particularly, approximately 30 mg of Genotropin was compacted under pressure using a Carver Laboratory Pellet Press (Model 3620, available from Carver, Inc., Wabash, IN). The pressure of compaction was 15,000 lbs/in², which was applied for approximately 45 seconds. A pellet was obtained which was ground using mortar and pestle until visually broken up. The resulting reduced pellet was then sieved using a 53 μm sieve (Endecott, London). Particles having a size greater than 53 μm were selected and appropriate dosages thereof were measured into drug cassettes for delivery from a needleless syringe.

Example 4
Visual Assessment of rhGH Particle Penetration

Lyophilized recombinant human growth hormone (rhGH) powder, and densified rhGH particles prepared as described in Example 3 were administered to porcine subjects using a needleless syringe. The degree of particle penetration was visually ascertained as follows.

Genotropin® 36 IU lyophilized powder was milled gently, weighed into individual doses of approximately 0.8 mg powder and loaded into a needleless syringe device for delivery. Densified Genotropin® was prepared as described in Example 3 and approximately 0.8 mg of the densified particles were loaded into a needleless syringe device for delivery.

Porcine subjects were prepared by clipping a sufficient area on the hindquarters. The lyophilized powder and densified particles were delivered to the porcine skin under high velocity. Upon a side-by-side comparison, it was observed that a higher proportion of the densified particles penetrated the skin as evidenced by the visual presence of the lyophilized powder remaining largely on the surface of the skin while substantially no densified particles were observed to remain on the surface of the skin.

Example 5
Serum Levels of Transdermally-Delivered rhGH

In order to determine the efficiency with which densified rhGH is delivered using a needleless syringe system, the following study was carried out. Three groups of 5 healthy New Zealand White rabbits were prepared by clipping the fur from the flank area to expose a sufficient area for delivery of lyophilized recombinant human growth hormone powder (rhGH) or densified rhGH by needleless syringe.

Approximately 0.8 mg of lyophilized Genotropin® powder was reconstituted into 1.8 mL of a suitable buffer without preservatives (e.g., sterile phosphate buffered saline (PBS)) to provide a Genotropin® solution having a concentration of 20 IU/mL. 1 mL dosages were withdrawn by syringe and gently mixed with 1 mL of buffer to

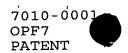provide an injection solution having a concentration of
10 IU/mL.

Each animal in the first group were given 0.1 mL/kg
of the injection solution by subcutaneous needle and

5   syringe injection, and the injection site was observed to
ensure that there was no leakage of the injected solution
after administration. Venous blood samples were taken
from the marginal ear vein of the right ear of each
animal at 0, 30 minutes, 1, 2, 4, 6, 12 and 24 hours

10   after administration. Serum levels of rhGH were
ascertained using an immunoassay with labeled anti-rhGH
antibodies. The mean serum levels of subcutaneously
delivered Genotropin® (•) found in the animals of Group 1
at each time point are depicted in Figure 4.

15   Approximately 2 mg of lyophilized Genotropin® powder
was loaded into a needleless syringe. The lyophilized
powder was administered to each animal in the second
group by needleless injection at high velocity. Venous
blood samples were taken from the marginal ear vein of

20   the right ear of each animal at 0, 30 minutes, 1, 2, 4,
6, 12 and 24 hours after administration. Serum levels of
the administered rhGH were ascertained using an
immunoassay with labeled anti-hGH antibodies. The mean
serum levels of transdermally injected lyophilized

25   Genotropin® powder (▲) found in the animals of Group 2
at each time point are depicted in Figure 4.

Approximately 2 mg of densified Genotropin®
particles prepared as in Example 3 was loaded into a
needleless syringe. The densified particles were

30   administered to each animal in the third group by
needleless injection at high velocity. Venous blood
samples were taken from the marginal ear vein of the
right ear of each animal at 0, 30 minutes, 1, 2, 4, 6, 12
and 24 hours after administration. Serum levels of the

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administered rhGH were ascertained using an immunoassay
with labeled anti-rhGH antibodies. The mean serum levels
of transdermally injected densified Genotropin® particles
(■) found in the animals of Group 3 at each time point

5   are depicted in Figure 4.

As can be seen, markedly increased blood serum
levels of the densified Genotropin® particles
administered by needleless syringe were obtained as
compared to the lyophilized Genotropin® powder.

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Example 6
Determination of Optimum Conditions
for Needleless Syringe Delivery of rhGH

In order to determine optimum conditions for

15   delivery of rhGH using a needleless syringe delivery
system, the following study is carried out. One group of
8 healthy New Zealand White rabbits (2 ± 0.25 kg) are
prepared by clipping the fur from the flank area to
expose a sufficient area for delivery of Lyophilized

20   recombinant human growth hormone powder (rhGH) or
densified rhGH by needleless syringe, sub-cutaneous (SC)
or intravenous (IV) injection. The animals are weighed
at the start of the study and on a weekly basis
throughout the study to determine appropriate Genotropin®

25   dosages. The animals remain in one large group to obtain
statistically-significant data.

For an initial needleless syringe injection series,
Genotropin® 36 IU lyophilized powder is milled gently and
filled into a glass vial. The milled lyophilized powder

30   is weighed into individual dosages and loaded into
needleless syringe devices at approximately 0.8 mg
powder/kg. The injection is conducted in the first week
of the study, and multiple venous blood samples (1 mL
whole blood) are taken from the marginal ear vein at

35   times 0, 30 minutes, 1, 2, 4, 6, 12, 24 and 48 hours

after administration.   The animals are individually-
housed at all times with food and water available *ad
libitum.*

5   Blood samples are handled and processed as follows:
each venous blood sample is allowed to clot at ambient
temperature for approximately 30 minutes and then left
for an additional 30 minutes at approximately 4 °C.
Clotted samples are centrifuged for 10 minutes and the
serum is aspirated and stored at -20 °C for analysis.

10   In the second week of the study, (approximately 1
week following the initial needleless injection), the
animals are administered a Genotropin® formulation
prepared as follows: 36 IU (approximately 30 mg) of the
lyophilized Genotropin® powder is reconstituted into 1.8

15 mL of a suitable buffer without preservatives (e.g.,
sterile phosphate buffered saline (PBS)) to provide a
Genotropin® solution having a concentration of 20 IU/mL.
1 mL dosages are withdrawn by syringe and gently mixed
with 1 mL of buffer to provide an injection solution

20 having a concentration of 10 IU/mL. Each animal is given
an IV injection of 0.1 mL/kg of the solution in the left
ear.

         Following IV injection, multiple venous blood
samples are taken from the marginal ear vein of the right

25 ear at times 0, 5, 10, 30 minutes, 1, 2, 4, 6 and 12
hours after injection.

         In the third week of the study, the animals are
administered 0.1 mL/kg of a buffered Genotropin® solution
(prepared as above) by sub-cutaneous injection. The

30 injection site is observed to ensure that there is no
leakage after administration. Following the SC
injections, multiple venous blood samples are taken from
the marginal ear vein of the right ear at times 0, 30
minutes, 1, 2, 4, 6, 12, 24 and 48 hours after

35 administration.

In the fourth week of the study, approximately 0.8 mg powder/kg of densified Genotropin® particles (prepared as described in Example 3) are administered to each animal using a needleless syringe, and multiple venous

5   blood samples are taken from the marginal ear vein of the right ear at times 0, 30 minutes, 1, 2, 4, 6, 12, 24 and 48 hours after administration.

Serum rhGH levels are determined as previously described and pharmacokinetic variables are calculated

10   for each drug administration technique. It is expected that needleless syringe administration of the densified Genotropin® particles will result in achieving and maintaining *in vivo* therapeutic levels of the administered drug.

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Example 7
Bio-Activity of Densified rhGH Delivered
In Vivo to Growth Hormone-Deficient Rats

To evaluate the bio-activity of recombinant human

20   growth hormone that has been densified using the method of the present invention the following study is carried out.

Dwarf or hypophysectomized (growth hormone-deficient) rats are administered pharmaceutical

25   preparations containing either: densified Genotropin® particles; lyophilized Genotropin® powder; or placebo at approximately 4 IU rhGH per animal/week by daily sub-cutaneous (SC) injection. In particular, on 5 successive days, fur from the peritoneal region of the animal

30   subjects is clipped prior to administration of the densified rhGH, the lyophilized rhGH or the placebo by SC injection. Body weight and, if desired, bone size and length are monitored on a daily basis.

Bio-activity of the densified rhGH particle

35   formulation is determined by measuring body weight change

over time. It is expected that the densified rhGH
particle formulation will retain sufficient bio-activity.

Example 8

Densification of Commonly Used Excipients

Finely ground powders of pharmaceutical grade
mannitol and lactose were obtained and reprocessed using
the method of the invention. Particularly, approximately
30 to 50 mg of mannitol or lactose were compacted under
pressure using a Carver Laboratory Pellet Press (Model
3620, available from Carver, Inc., Wabash, IN). The
pressure of compaction was 10,000 lbs/in^2 which was
applied for approximately 30 seconds. The resulting
compacted pellets were ground using mortar and pestle
until visually broken up, and then sieved to select for
particles having a size greater than about 50 μm using
the methods described in Example 3. In both the mannitol
and the lactose preparations, a significant size
reduction was observed when the compacted particles were
compared against like weights of the non-densified
starting materials.

Example 9

Quantification of Densified Excipients

Pharmaceutical grade trehalose and mannitol
excipients were obtained and processed according to the
method of the invention. Both excipient preparations
were processed in several different ways, and absolute
density, envelope density, and average particle size of
the resultant preparations were measured as described
below.

Trehalose 45H3830 (Sigma) and mannitol K91698380-703
(Merck) were either sieved, or freeze dried, compacted,
ground and then sieved. A range of the resulting

preparations were then analyzed for particle size and density measurements.

Portions of each sugar excipient were sieved to obtain preparations having reduced particle sizes.

5   Sieving was carried out using stainless steel sieves for two hours at 3 mm amplitude using three sieve sizes (75μm, 53 μm and 38 μm) without additional processing.

Alternatively, portions of each sugar excipient were processed using the methods of the invention as follows.

10   40g of each of the sugars was dissolved in water, flash frozen, and then freeze dried over night. Samples of each freeze dried preparation were retained, and the remainder compacted in a 13 mm compression die (15,000 lbs/inch^2 for 45 seconds) into discs. The mannitol discs

15   were ground using mortar and pestle, and then sieved as described above at 3 mm amplitude, using three sieve sizes. The trehalose discs were first ground in a vibratory ball mill (Retsch mill), then ground by mortar and pestle and sieved as above.

20   Samples from each of the above-described excipient preparations were then analyzed for absolute and envelope density. Absolute density was determined using helium pycnometry, and envelope density was determined using a GeoPyc™ Model 1360 Envelope Density Analyzer

25   (Micromeritics Instrument Corp.). The results of the analysis are depicted below in Table 4.

30

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Table 4				
Pretreatment	Trehalose Density (g/cm^3)		Mannitol Density (g/cm^3)	
	Absolute	Envelope	Absolute	Envelope
Sieved	1.5	0.5	1.5	0.5
Freeze Dried	1.5	0.3	1.5	0.3
Freeze Dried, Compressed, Milled, Sieved	1.5	0.8	1.5	0.8

As can be seen in Table 4, none of the various processing methods had a significant effect on the absolute density of the powdered excipients. Further, as expected, the non-compacted, freeze-dried sugars had a much lower envelope density than the other preparations, and a concomitantly higher porosity (measurement not shown). The density measurements for the trehalose and mannitol samples clearly demonstrate that the methods of the invention (compression, milling, sieving) lead to a significant increase in envelope density relative to both the freeze-dried and the sieved preparations. These results indicate that the novel methods described herein can be used to provide densified particulate pharmaceutical preparations that are suitable for delivery via needleless injection techniques.

Accordingly, novel methods for DNA delivery as well novel methods for densifying particulate pharmaceutical compositions, and densified pharmaceutical compositions formed therefrom, have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit

and the scope of the invention as defined by the appended claims.

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